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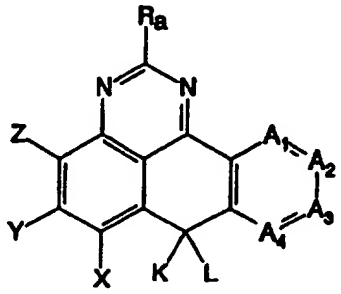
(54) Title: BENZOPERIMIDINE-CARBOXYLIC ACIDS AND DERIVATIVES THEREOF

(57) Abstract

Benzoperimidine-carboxylic acids and derivatives of general structural formula (I) wherein R, A, B, C, D, K, L, X, Y, and Z are as defined, having activity for receptors of corticotropin releasing factor (CRF). The compounds are useful in treating stress-related diseases, cardiovascular, neurological and psychiatric disorders including anxiety, depression, eating disorders, anorexia nervosa, superanuclear palsy, irritable bowel syndrome, gastrointestinal diseases, immune suppression, inflammatory disorders, drug and alcohol withdrawal symptoms, drug addiction, Alzheimer's disease or fertility disorders.



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BENZOPERIMIDINE-CARBOXYLIC ACIDS AND DERIVATIVES THEREOF

Field of the Invention

This invention relates to non-peptidic antagonists of corticotropin releasing factor receptors. It relates particularly to perimidine-based compounds having CRF antagonist activity.

Background of the Invention

CRF is a 41-amino acid linear peptide isolated from ovine hypothalmia. CRF plays a crucial role in integrating the body's overall response to stress. Although the existence of CRF was postulated more than thirty years ago (G.W. Harris, *Physiol. Rev.* 28:139), its purification and sequencing was reported in 1981 (W. Vale *et al.*, *Science*, 213, 1394 (1981); J. Spiess *et al. Proc. Natl. Acad. Sci., U.S.A.*, 78, 6517 (1981)). Shortly thereafter the sequences of human and rat CRF were determined and these were found to be the same, but differed from ovine CRF (oCRF) in 7 of the 41 amino acid residues (J. Rivier *et al. Proc. Natl. Acad. Sci., U.S.A.*, 80, 4851 (1983)); Furutani *et al. Nature*, 301, 537 (1983). CRF produces profound alterations in behavioral and autonomic nervous system functions (M.R. Brown and L.A. Fisher, *Fed. Proc.*, 44, 243 (1985); G. F. Koob, F. E. Bloom, *Fed. Proc.*, 44, 259 (1985)). Upon direct administration into the brain, CRF initiates behavioral, physiological and endocrine responses that are essentially identical to those observed when animals are exposed to stressful environment. When given, for example, by intracerebroventricular (icv) injection, CRF induces behavioral activation (R.E. Sutton *et al. Nature* 297, 331 (1982)), it produces a long-lasting activation of the electroencephalogram (C.L. Ehlers, *et al. Brain Res.* 278, 332 (1983)), stimulates the sympathoadrenomodullary pathway (M.R. Brown *et al. Endocrinology* 110, 928 (1982)), increases heart rate, raises blood pressure and increases oxygen consumption (L.A. Fisher *et al. Endocrinology* 110, 2222 (1982)), alters gastrointestinal activity (M. R. Brown *et al. Life Sciences* 30, 207 (1982), suppresses food intake (C. L. Williams *et al. Am. J. Physiol.*, 253, G582 (1987) and sexual behavior (A.S. Levine *et al. Neuropharmacology*, 22, 337 (1983)), and affects immune function (D. J. S. Sirinathsinghji *et al. Nature*, 305, 232 (1983); M. Irwin et al. *Am. J. Physiol.* 225, R744 (1988).

The actions of CRF in the peripheral and central nervous system are media ted through multiple binding sites. These CRF binding sites are heterogeneous with respect to sequence, pharmacology, and tissue distribution. Three CRF receptors, CRF_1, $CRF_{2\alpha}$ and $CRF_{2\beta}$, which encode 411-, 415-, and 431-amino acid proteins respectively, have been reported to date. The reported CRF receptors comprise seven putative membrane-spanning domains characteristic of G_S-coupled receptors. All three CRF receptors transduce a signal which involves stimulation of cAMP production.

A few classes of non-peptide CRF receptor antagonists have been reported in the past few years. Derivatives of 4-substituted thio-5-oxo-3-pyrazolines have been disclosed as CRF antagonists in US patent 5,420,133. A weakly potent class of CRF antagonists has been reported in European patent application EP 0576350A1 (1993). Series of patent applications (WO 94/13643, WO 94/136344, WO 94/13661 and WO 94/13677) claiming non-peptide compounds as CRF antagonists have been reported by Pfizer and Co., Inc. The duPont Merck pharmaceutical

company has recently disclosed a class of CRF antagonists, 1N-alkyl-N-arylpyrimidines and their derivatives in international patent application WO 95/10506.

Summary of the Invention

According to the invention there are provided CRF ligands having structures defined by the general Formula I. The invention includes compounds used as intermediates in the preparation of the product compounds. Preferred intermediate compounds are those wherein the substituents K and L together include a carbonyl group. Preferred compounds are those whose synthesis is disclosed in Examples 3, 4, 7, 11, 12, 13, and 15-17.

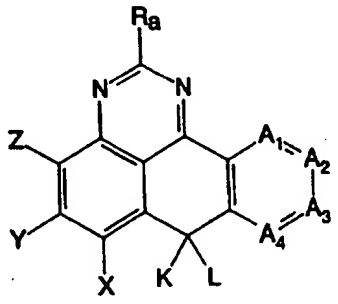
According to another aspect of the invention there are provided methods for synthesis of the claimed compounds. According to yet another aspect of the invention there are provided pharmaceutical formulations comprising the compounds of the invention.

The invention also includes methods of treatment for diseases and disorders, including stress-related diseases, cardiovascular, neurological and psychiatric disorders including anxiety, depression, eating disorders, anorexia nervosa, superanuclear palsy, irritable bowel syndrome, gastrointestinal diseases, immune suppression, inflammatory disorders, drug and alcohol withdrawal symptoms, drug addiction, Alzheimer's disease or fertility disorders by administration of the compounds disclosed.

Detailed Description of the Preferred Embodiment

Substances which specifically inhibit the binding of CRF to its receptors are believed to block the physiological effects of CRF and these chemical entities would be useful in treating patients with CRF related disorders. The present invention discloses potent CRF receptor antagonists that are non-peptidic small molecules structurally distinct from those previously reported.

The compounds of the invention are of general formula I:



I

wherein R_a is:

(a) H;

(b) (C_1-C_6)alkyl groups which are linear or branched, saturated or unsaturated and optionally substituted with amine, hydroxyl, halogen or carboxyl groups;

(c) allyl;

(d) (C_3-C_6)-cycloalkyl;

(e) aryl, wherein aryl is defined as phenyl or naphthyl unsubstituted or substituted with 1 or 2 or 3 substitutents selected from the group consisting of:

(i) (C_1-C_6) alkyl;

(ii) (C_3-C_7) alkenyl;

(iii) (C_3-C_7) cycloalkyl;

(iv) (C_1-C_6) alkoxy;

(v) F; Cl; Br; I;

(vi) NO_2;

(vii) CN;

(viii) NH_2;

(ix) $NHCO(C_1-C_6)$ alkyl;

(x) $NH(C_3-C_6)$ cycloalkyl;

(xi) CO_2H;

(xii) $CO_2(C_1-C_6)$alkyl;

(xiii) $CO_2(C_3-C_6)$cycloalkyl;

(xiv) $NHCONH(C_1-C_6)$alkyl;

(xv) $NHCO(C_3-C_6)$cycloalkyl;

(xvi) $NHSO_2$-(C_1-C_6)alkyl;

(xvii) $NHSO_2$-(C_3-C_6)cycloalkyl;

(xviii) $CONHSO_2$-(C_1-C_6)alkyl;

(xix) $CONHSO_2$-(C_3-C_6)cycloalkyl;

(xx) $CONHSO_2$-aryl;

(xxi) OH;

(xxii) OCO-(C_1-C_6)alkyl;

(xxiii) OCO-(C_3-C_6)cycloalkyl;

(xxiv) OCO-aryl;

(xxv) CF_3;

(xxvi) (C_1-C_4)alkylthio; or

(f) heteroaryl, wherein heteroaryl is defined as an unsubstituted, monosubstituted or disubstituted heteroaromatic 5- or 6-membered cyclic moiety, which can contain one or two members selected from the group consisting of N, O, S and wherein the substitutents are members selected from the group consisting of:

(i) Cl; Br; I; or F;

(ii) OH;

(iii) SH;

(iv) NO_2;

(v) NH_2;

(vi) $NH(C_1\text{-}C_6)$alkyl, or $NH(C_1\text{-}C_6)_2$alkyl;

(vii) $(C_1\text{-}C_5)$alkyl;

(viii) $(C_1\text{-}C_5)$alkoxy;

(ix) $(C_1\text{-}C_4)$-perfluoroalkyl; CF_3;

(x) $(C_2\text{-}C_4)$alkenyl;

(xi) $(C_2\text{-}C_4)$alkynyl;

Z is:

(a) H;

(b) CO_2H;

(c) $CO_2\text{-}(C_1\text{-}C_6)$alkyl;

(d) $CONH_2$;

(e) $CONH\text{-}(C_1\text{-}C_8)$alkyl;

(f) $CON\text{-}((C_1\text{-}C_8)\text{alkyl})_2$;

(g) $CONH\text{-}(C_1\text{-}C_8)\text{alkyl}_2$;

(h) $CONH\text{-}(C_1\text{-}C_8)\text{alkyl-NH-}(C_1\text{-}C_4)$alkyl;

(i) $CONH\text{-}(C_1\text{-}C_8)\text{alkyl-NH-}((C_1\text{-}C_4)\text{alkyl})_2$;

(j) $CONH\text{-}(C_1\text{-}C_8)$cycloalkyl;

(k) $CONH\text{-}(C_1\text{-}C_8)\text{cycloalkyl-}NH_2$;

(l) $CONH\text{-}(C_1\text{-}C_8)\text{cycloalkyl-NH-}(C_1\text{-}C_4)$alkyl;

(m) $CONH\text{-}(C_1\text{-}C_8)\text{cycloalkyl-N-}((C_1\text{-}C_4)\text{alkyl})_2$;

(n) $CONH\text{-}(C_1\text{-}C_3)\text{alkyl-}(C_1\text{-}C_8)\text{cycloalkyl-}(C_1\text{-}C_3)\text{alkyl-}NH_2$;

(o) $CONH\text{-}(C_1\text{-}C_3)\text{alkyl-}(C_1\text{-}C_8)\text{cycloalkyl-}(C_1\text{-}C_3)\text{alkyl-NH-}(C_1\text{-}C_3)$alkyl;

(p) $NHCO\text{-}(C_1\text{-}C_8)\text{alkyl-}NH_2$;

(q) $NHCO\text{-}(C_1\text{-}C_8)$-cycloalkyl;

(r) $NHCO\text{-}(C_1\text{-}C_8)\text{-cycloalkyl-}NH_2$;

(s) $CONHNH(C_1\text{-}C_8)\text{alkyl-}NHNH_2$;

(t) $CONHNH(C_1\text{-}C_8)\text{cycloalkyl-}NHNH_2$;

(u) CN;

(v) NO_2;

(w) CHO;

(x) $SO_2NH\text{-}(C_1\text{-}C_8)\text{alkyl-}NH_2$;

(y) $SO_2NH\text{-}(C_1\text{-}C_8)\text{-cycloalkyl-}NH_2$;

(z) NH-SO_2-(C_1-C_8)alkyl-NH_2;

(aa) NH-SO_2-(C_1-C_8)cycloalkyl-NH_2;

(ab) imidazole and optionally C2-substituted derivatives thereof;

(ac) imidazoline and substituted derivatives thereof;

(ad) indole and substituted derivatives thereof;

(ae) piperazine and substituted derivatives thereof;

wherein the substitutents of groups (ab)-(ae) are selected from the group consisting of alkyl halides; carboxylic acids, amides and alkyl esters;

X and Y are independently:

(a) H;

(b) NH(C_1-C_6)alkyl, straight or branched C-C chain;

(c) N((C_1-C_6)$alkyl_2$), straight or branched C-C chain;

(d) NH(C_1-C_6)alkyl-NH_2, straight or branched C-C chain;

(e) N((C_1-C_6)alkyl-NH_2)$_2$, straight or branched C-C chain;

(f) CN;

(g) (C_1-C_6)alkyl-NH(C_1-C_6)alkyl;

(h) (C_1-C_6)alkyl-O-(C_1-C_6);

(i) NO_2;

(j) -amidine;

(k) mono- and di-substituted amidines;

(l) guanidines;

(m) mono- and di-substituted guanidines;

(n) (C_1-C_6)NH_2;

(o) NHCO-(C_1-C_6)alkyl, NHCO-aryl, NHCO-heteroaryl;

(p) NHCO-O(C_1-C_6)alkyl, NHCO-O-aryl, NHCO-O-heteroaryl;

(q) NHCONH(C_1-C_6)alkyl, NHCONH-aryl, NHCONH-heteroaryl;

(r) CONH(C_1-C_6)alkyl-NH_2;

(s) CONH(C_3-C_6)cycloalkyl-NH_2;

or alternatively, X and Y are joined to form one or two rings (ring size = 5,6,7,8 membered cycloalkanes) through N or O or S;

X and Y are also independently:

(a) NH-(CH_2)n-NH- (n = 2,3,4,5,6,7,8);

(b) 1,2-diamino-cycloalkanes and substituted derivatives thereof;

(c) 1,2-diamino-cyclopropane and substituted derivatives thereof;

(d) 1,2-diamino-cyclobutane and substituted derivatives thereof;

(e) 1,2-diamino-cyclopentane and substituted derivatives thereof;

(f) 1,2-diamino-cyclohexane and substituted derivatives thereof;

(g) 1,2-diamino-cycloheptane and substituted derivatives thereof;

(h) 1,2-diamino-cyclooctane and substituted derivatives thereof;

(i) 1,2-phenylene diamine (1,2-diaminobenzene) and substituted derivatives thereof;

(j) 2,3-diaminopyridine;

K and L are independently:

(a) H;

(b) =O;

(c) OH;

(d) $(C_1\text{-}C_3)$alkyl;

(e) O-$(C_1\text{-}C_3)$alkyl;

(f) CH_2;

(g) CN;

(h) CO_2-alkyl;

(i) CO_2H;

(j) -N-alkyl;

(k) -N-aryl;

(l) -N-heteroaryl;

(m) $-CHCO_2H$;

(n) -CHCONH-alkyl;

(o) $-CHCO_2$alkyl;

wherein the substituents of X, Y, K and L are selected from the substituent groups defined for R(b); R (e); R(f) and R(ab)-(ae); and

A1, A2, A3, and A4 are independently CH;

The invention also includes all enantiomers and stereoisomers of these compounds, and pharmaceutically acceptable salts thereof. A pharmaceutically acceptable salt is a physiologically non-toxic salt that does not interfere with the pharmacologic action of the CRF ligands of the invention. Suitable salts are disclosed in Berge. S.M. (1977) J. Pharmaceut. Sci. 66(1): 1-18.

Preferred compounds are benzoperimidine derivatives wherein X and Y of the general formula are incorporated into a diamino-cyclohexane ring system. Also preferred is a Z substituent comprising a carboxyl amide. Preferred compounds are also benzoperimidine derivatives wherein X and Y taken together form a 5,6, fused piperazine ring. Also preferred are benzoperimidine derivatives wherein A1-A2-A3-A4 forms a ring system that may

be pyrrole, thiophene, furan, oxazole, isoxazole, thiazole, imidazole, or isothiazole. A ketone at K,L is a preferred structure for the chemical synthesis of these compounds.

The compounds disclosed herein are specifically designed as pharmacological agents, useful for, but not limited to, the treatment of the following conditions: stress-related diseases, cardiovascular, neurological and psychiatric disorders including anxiety, depression, eating disorders, anorexia nervosa, superanuclear palsy, irritable bowel syndrome, gastrointestinal diseases, immune suppression, inflammatory disorders, drug and alcohol withdrawal symptoms, drug addiction, Alzheimer's disease or fertility disorders.

The compounds according to the invention, which may also be referred to as active ingredients, may be administered for therapy by any suitable route including oral, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous and intradermal). It will be appreciated that the preferred route will vary with the condition and age of the recipient, the nature of the condition to be treated, and the chosen active ingredient.

The dosage for therapeutic use of the disclosed compounds can be determined using conventional considerations, including the clinical indication. It will be appreciated that the actual preferred amounts of active compound administered in a specific case will also vary according to specific active agent, the formulation, the route of administration. In general, a dose will be in the range of 0.1 to 120 mg per kilogram body weight of the recipient per day. The desired dose is preferably presented as two, three or more subdoses administered in unit dosage form.

For parenteral routes, such as intravenous, intrathecal, intramuscular and similar administration, typically doses are lower than the dose employed for oral administration.

The compounds may be administered alone or in combination with pharmaceutically acceptable carriers, in either single or multiple doses. Pharmaceutically acceptable carriers are defined as substances that are not toxic to the patient and that do not destroy the activity of the active compound. Pharmaceutical preparations containing the compounds of the invention in combination with various carriers are produced by conventional dissolving and lyophilizing processes to contain from approximately 0.1% to 100%, preferably from approximately 1% to 50% of the active ingredient. They can be prepared as ointments, salves, tablets, capsules, powders or sprays, together with effective excipients, vehicles, diluents, fragrances or flavor to make palatable or pleasing to use.

For parenteral administration, solutions of the novel compounds of formula I in sterile aqueous solution, aqueous propylene glycol or sesame or peanut oil may be employed. Such aqueous solutions should be suitable buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. The aqueous solutions are particularly suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. The sterile aqueous media employed are all readily available by standard techniques known to those skilled in the art. Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solution and various organic solvents. The pharmaceutical compositions formed by combining the compounds of formula I and the pharmaceutically acceptable carriers are then readily administered in a variety of dose forms suitable for the disclosed

routes of administration. The formulations may conveniently be presented in unit dosage form by methods known in the art of pharmacy.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules or tablets, each containing a predetermined amount of the active ingredient in the form of powder or granules, as a solution or suspension in an aqueous or nonaqueous liquid, or as an oil-in-water or water in oil liquid emulsion.

Tablets or other non-liquid oral compositions may contain acceptable excipients, known to the art for the manufacture of pharmaceutical compositions, comprising diluents, such as lactose or calcium carbonate; binding agents such as gelatin or starch; and one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring or preserving agents to provide a palatable preparation. Moreover, such oral preparations may be coated by known techniques to further delay disintegration and absorption in the intestinal tract.

Aqueous suspensions may contain the active ingredient in admixture with pharmacologically acceptable excipients, comprising suspending agents, such as methyl cellulose; and wetting agents, such as lecithin or long-chain fatty alcohols. The said aqueous suspensions may also contain preservatives, coloring agents, flavoring agents and sweetening agents in accordance with industry standards.

Preparations for topical and local application comprise aerosol sprays, lotions, gels and ointments in pharmaceutically appropriate vehicles which may comprise lower aliphatic alcohols, polyglycols such as glycerol, polyethylene glycol, esters of fatty acids, oils and fats, and silicones. The preparations may further comprise antioxidants, such as ascorbic acid or tocopherol, and preservatives, such as p-hydroxybenzoic acid esters.

Parenteral preparations comprise particularly sterile or sterilized products. Injectable compositions may be provided containing the active compound and any of the well known injectable carriers. These may contain salts for regulating the osmotic pressure. Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solution and various organic solvents. The pharmaceutical compositions formed by combining the novel compounds of formula I and the pharmaceutically acceptable carriers are then readily administered in a variety of dosage forms suitable for the disclosed routes of administration. The formulations may conveniently be presented in unit dosage form by methods known in the art of pharmacy.

The compounds of the invention are prepared as described below and are readily screened for receptor-binding using the binding assay of Example 32 and the biological assays of Examples 33-34.

The preparation of the compounds of the present invention is described in detail using the following examples, but the chemical reactions described are disclosed in terms of their general applicability to the preparation of the ligands of the invention. Occasionally, the reaction may not be applicable as described to each compound included within the disclosed scope of the invention. The compounds for which this occurs will be readily recognized by those skilled in the art. In all such cases, either the reactions can be successfully performed by conventional modifications known to those skilled in the art, that is, by appropriate protection of interfering groups, by changing to alternative conventional reagents, or by routine modification of reaction conditions. Alternatively, other reactions disclosed herein or otherwise conventional will be applicable to the preparation of the

corresponding compounds of the invention. In all preparative methods, all starting materials are known or readily preparable from known starting materials; all temperatures are set forth in degrees Celsius; and, unless otherwise indicated, all parts and percentages are by weight.

SYNTHESIS

The preparation of the compounds of the present invention is described in detail using the following examples, but the chemical reactions described are disclosed in terms of their general applicability to the preparation of the ligands of the

invention. Occasionally, the reaction may not be applicable as described to each compound included within the disclosed scope of the invention. The compounds for which this occurs will be readily recognized by those skilled in the art. In all such cases, either the reactions can be successfully performed by conventional modifications known to those skilled in the art, that is, by appropriate protection of interfering groups, by changing to alternative conventional reagents, or by routine modification of reaction conditions. Alternatively, other reactions disclosed herein or otherwise conventional will be applicable to the preparation of the corresponding compounds of the invention. In all preparative methods, all starting materials are known or readily preparable from known starting materials; all temperatures are set forth in degrees Celsius; and, unless otherwise indicated, all parts and percentages are by weight.

The benzoperimidine derivatives of the general structure I were prepared by the general schemes 1 to 5 shown below. A representative example of the general structure I, where R = H; Z = CO_2H; X and Y = H; K and L = (=O) and A1, A2, A3, A4 each are C, was used as a starting material to synthesize the derivative products. Similar reaction sequence(s) may be applied towards the synthesis of other derivatives.

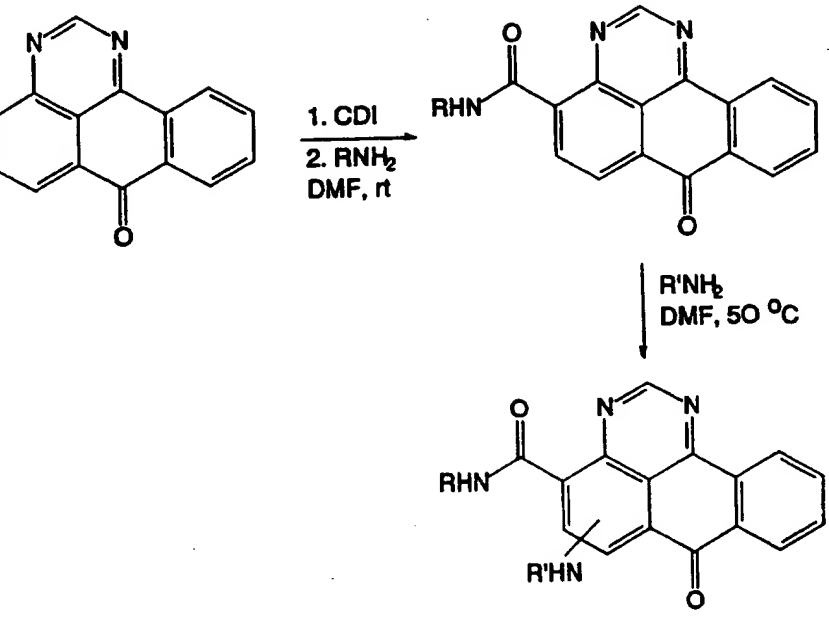
SCHEME 1

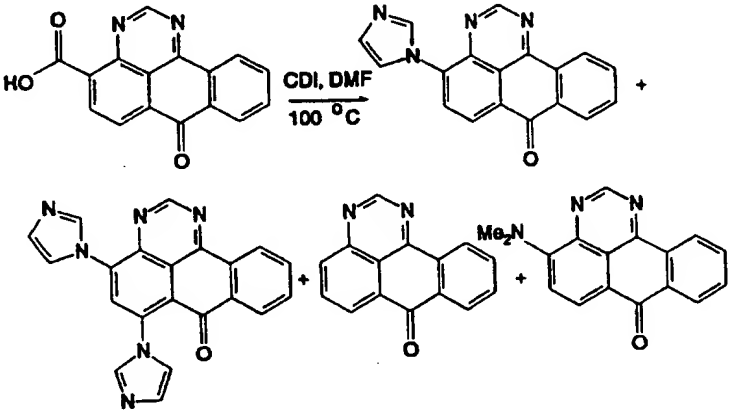
Method A involves conversion of the carboxylic group to amide in step 1 using 1, 1'-carbonyldiimidazole (CDI) as a coupling agent in DMF as a solvent at room

temperature. The resulting amide was further treated in step 2 with a different amine or diamine to give the product as shown below.

SCHEME 2

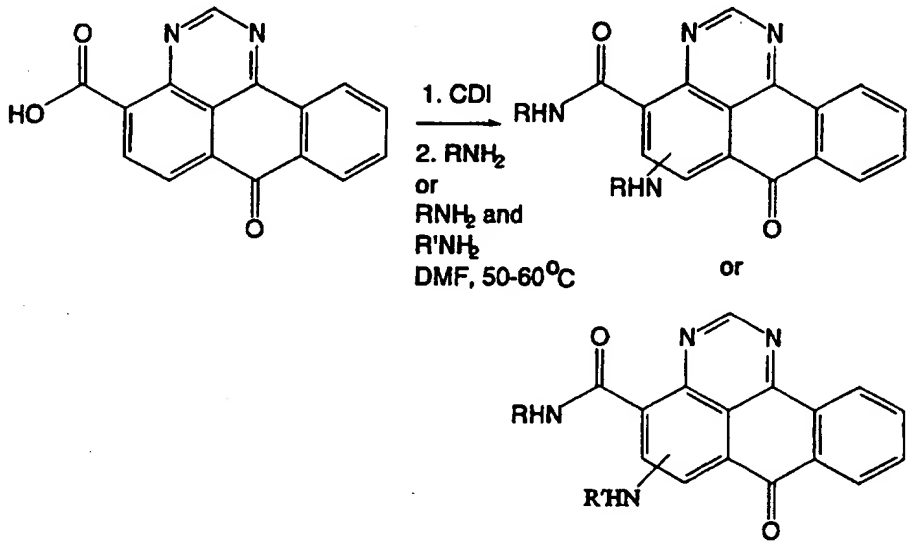
In Method B, 7-oxo-benzo[e]perimidine-4-carboxylic acid was treated with 1, 1'-carbonyldiimidazole in DMF at 100°C. Four different products, besides the expected imidazole amide were isolated as shown below.





SCHEME 3

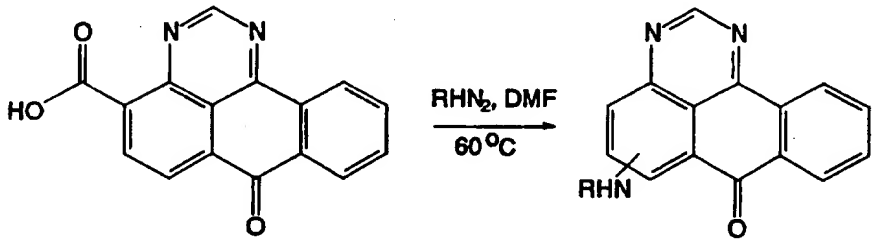
Method C involves the synthesis of derivatives of 7-oxo-benzo[e]perimidine-4-carboxylic acid by using one particular amine or diamine or a mixture of two different amines or diamines in the presence of 1,1'-carbonyldiimidazole as shown below.



SCHE

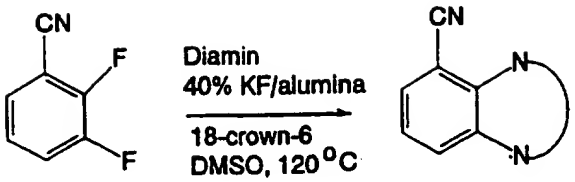
ME 4

In method D, 7-oxo-benzo[e]perimidine-4-carboxylic acid was treated with an amine in the absence of 1,1'-carbonyldiimidazole for 48 h at 60°C. A decarboxylated product was isolated as shown below.



SCHEME 5

Derivatives similar to examples 30 and 31 can be prepared by the treatment of ortho disubstituted fluorobenzonitriles with diamines in DMSO in the presence of 40% KF/alumina and catalytic amount of 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane, Catalog No. 18,665-1, Aldrich-Milwaukee, WI) at 120°C as shown below.


CN
F
F
Diamin
40% KF/alumina
18-crown-6
DMSO, 120 °C
CN
N
N

General methods for preparation of compounds of general formula I

METHOD A

Step 1

To a solution of 0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid in 15 mL of dry DMF at 20°C was added 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole and the reaction mixture was stirred for 60 min. Next a solution of 1 mmol of the corresponding amine or diamine in 3 mL of dry DMF was added. The reaction was stirred for 18 h and final product was chromatographed on Waters Prep L.C. 4000 System: column PrePakR cartridge VydacTM C_18 (47 x 300 mm); UV absorbance, 2.0 AUFS @ 230; buffer A 0.1 % TFA; buffer B , 0.1% TFA in 60% CH_3CN/40% H_2O; flow rate, 95 mL/min; gradient 20% B to 90% B in 40 minutes.

Step 2

To a solution of 0.1 mmol of 7-oxo-7H-benzo[e]perimidine-4-carboxamide derivative (step A) in 10 mL of dry DMF was added a solution of 1 mmol of the corresponding diamine in 2 mL of dry DMF. The reaction was stirred for 6 h at 50°C and product was chromatographed on Waters Prep L.C. 4000 System: PrePak^R cartridge Vydac^{TM} C_18 (47 x 300 mm); UV absorbance, 2.0 AUFS @ 230; buffer A, 0.1 % TFA; buffer B , 0.1% TFA in 60% CH_3CN/40% H_2O; flow rate, 95 mL/min; gradient 20% B to 100% B in 40 min.

METHOD B

To a solution of 0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid in 20 mL of dry DMF was added 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole and the reaction mixture was stirred for 18 h at 100°C. Product was chromatographed on Waters Prep L.C. 4000 System: column, PrePak^R cartridge Vydac^{TM} C_18 (47 x 300 mm); UV absorbance, 2.0 AUFS @ 230; buffer A, 0.1 % TFA; buffer B , 0.1% TFA in 60% CH_3CN/40% H_2O; flow rate, 95 mL/min; gradient 20% B to 85% B in 60 min.

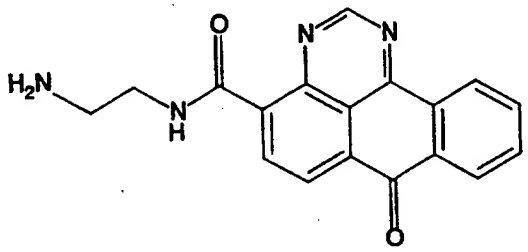
METHOD C

To a solution of 0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid in 15 mL of dry DMF at 20°C was added 0.162 g (1 mmol) of 1,1' carbonyldiimidazole and the reaction mixture was stirred for 60 min. Next a solution of 1 mmol of the corresponding amine or diamine in 3 mL of dry DMF was added. The reaction was stirred for 18 h at 50 - 60°C and chromatographed on Waters Prep L.C. 4000 System: column, PrePak^R cartridge Vydac^{TM} $C_1$8 (47 x 300 mm); UV absorbance, 2.0 AUFS @ 230; buffer A, 0.1 % TFA; buffer B , 0.1% TFA in 60% CH_3CN/40% H_2O; flow rate, 95 mL/min; gradient 20% B to 90% B in 40 min.

METHOD D

To a solution of 1 mmol of 7-oxo-7H-benzo[e]perimidine-4-carboxamide in 10 mL of dry DMF was added a solution of 1 mmol of the corresponding amine or diamine in 5 mL of dry DMF. The reaction was stirred for 48 h at 60°C and product was chromatographed on Waters Prep L.C. 4000 System: column, PrePak^R cartridge Vydac^{TM} C18 (47 x 300 mm); UV absorbance, 2.0 AUFS @ 230; buffer A, 0.1 % TFA; buffer B, 0.1% TFA in 60% CH_3CN/40% H_2O; flow rate, 95 mL/min.; gradient 10% B to 90% B in 40 min.

EXAMPLE 1

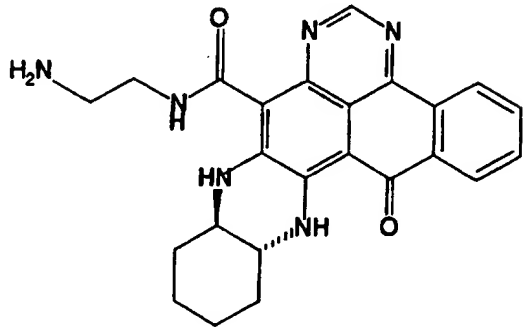


Preparation:

0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole and 0.062 g (1 mmol) of ethylenediamine and the reaction mixture was chromatographed (method A, step 1) to afford 0.222 g (70%) of the title compound: mp 208-210°C; ^{1}H NMR (300 MHz,

DMSO-d_6) δ 10.28-10.32 (m, 1H), 9.59 (s, 1H), 8.75-8.78 (m, 2H), 8.53-8.58 (m, 1H), 8.25-8.30 (m, 1H), 7.86-8.00 (m, 5H), 3.69-3.74 (m, 2H), 3.09-3.14 (m, 2H); APCIMS *m/z* 319 (M+1).

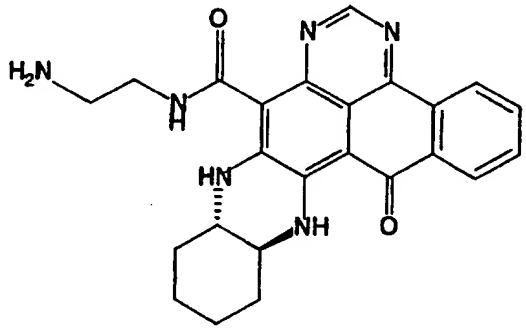
EXAMPLE 2



Preparation:

The product of example 1 (0.031 g, 0.1 mmol) was treated with 0.114 g (1 mmol) of (1R,2R)-(-)-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 2) to afford 0.033 g (80%) of the title compound: mp 128-132°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.94 (s, 1H), 11.75 (s, 1H), 11.20 (s, 1H), 8.99 (s, 1H), 8.76-8.83 (m, 1H), 8.67-8.75 (m, 1H), 8.30 (d, J = 7.64, 1H), 8.23 (d, J = 7.58, 1H), 7.75-7.97 (m, 7H), 3.57-3.67 (m, 2H), 3.48-3.56 (m, 1H) 3.13-3.23 (m, 1H), 3.02-3.12 (m, 2H) 2.10-2.23 (m, 1H), 2.00-2.10 (m, 1H)1.77-1.88 (m, 2H) 1.35-1.52 (m, 4H); APCIMS *m/z* 429 (M+1).

EXAMPLE 3

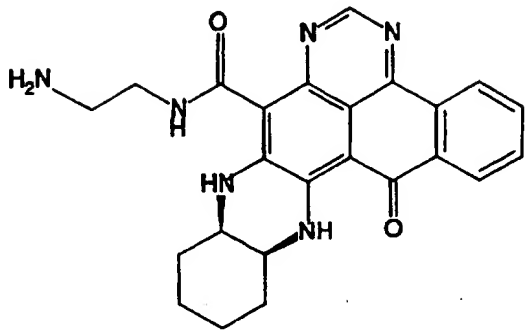


Preparation:

The product of example 1 (0.031 g, 0.1 mmol) was treated with 0.114 g (1 mmol) of (1S,2S)-(+)-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 2) to afford 0.032 g (78%) of the title compound: mp 228-230°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.01 (s, 1H), 11.78 (brs, 1H), 11.23 (s, 1H), 9.05 (s, 1H), 8.84 (d, J = 7.62, 1H), 8.35 (d, J = 7.57, 1H), 7.78-7.95 (m, 5H), 6.56 (brs. 1H) 3.55-3.74 (m, 2H),

3.00-3.25 (m, 4H), 2.13-2.25 (m, 1H), 2.03-2.12 (m, 1H), 1.75-1.90 (m, 2H), 1.35-1.55 (m, 4H); APCIMS *m/z* 429 (M+1).

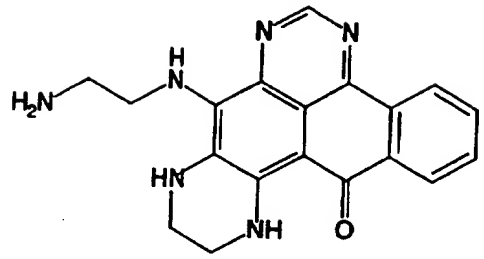
EXAMPLE 4



Preparation:

The product of example 1 (0.031 g, 0.1 mmol) was treated with 0.114 g (1 mmol) of cis-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 2) to afford 0.039 g (90%) of the title compound: mp 138-140°C; APCIMS *m/z* 429 (M+1).

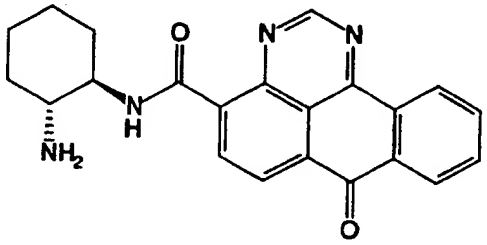
EXAMPLE 5



Preparation:

0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole, 0.062 g (1 mmol) of ethylenediamine and 0.114 g (1 mmol) of cis-1,2-diaminocyclohexane, the reaction mixture was chromatographed (method C) to afford 0.010 g (2.9%) of the title compound: mp 132-134°C; APCIMS *m/z* 347 (M+1).

EXAMPLE 6

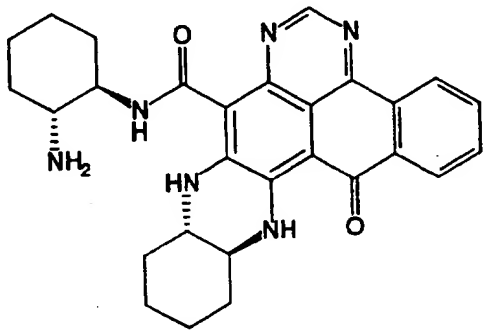


Preparation:

0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole and 0.114 g (1 mmol) of

(1R,2R)-(-)-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 1) to afford 0.268 g (72%) of the title compound: mp 167-170°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.19 (d, J = 8.73, 1H), 9.56 (s, 1H), 8.71-8.77 (m, 2H), 8.53 (d, J = 7.66, 1H), 8.23 (d, J = 7.65, 1H), 8.16 (brs, 2H), 7.91-7.98 (m, 1H), 7.83-7.89 (m, 1H), 4.05-4.16 (m, 1H) 3.18-3.31 (m, 1H), 2.04-2.17 (m, 2H), 1.75-1.90 (m, 2H), 1.45-1.70 (m, 2H), 1.32-1.45 (m, 2H); APCIMS *m/z* 373 (M+1).

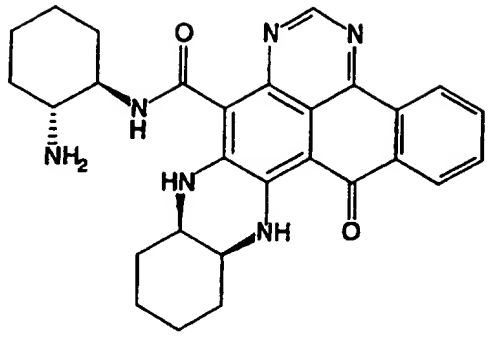
EXAMPLE 7



Preparation:

The product of example 6 (0.037, 0.1 mmol) was treated with 0.114 g (1 mmol) of (1S,2S)-(+)-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 2) to afford 0.038 g (80%) of the title compound: mp 165-167°C; ^{1}H NMR (300 MHz, DMSO-d_6) d 12.06 (s, 1H), 11.67 (d, J = 8.42,1 1H) 10.89 (s, 1H) 9.13 (s, 1H), 8.90 (d, J = 7.24, 1H), 8.41 (d, J = 6.91, 1H), 7.97 (brs, 2H), 7.90-7.96 (m, 1H), 7.83-7.88 (m, 1H), 3.97-4.10 (m, 1H), 3.32-3.43 (m, 1H), 3.11-3.27 (m, 2H), 2.17-2.27 (m, 1H), 1.98-2.10 (m, 3H), 1.73-1.90 (m, 4H), 1.30-1.70 (m, 8H); APCIMS *m/z* 483 (M+1).

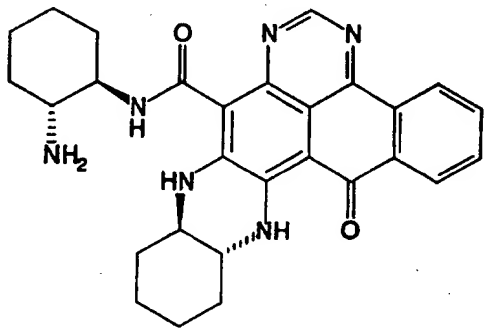
EXAMPLE 8



Preparation:

The product of example 6 (0.037, 0.1 mmol) was treated with 0.114 g (1 mmol) of cis-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 2) to afford 0.043 g (89%) of the title compound: mp 184-186°C; APCIMS *m/z* 483 (M+1).

EXAMPLE 9

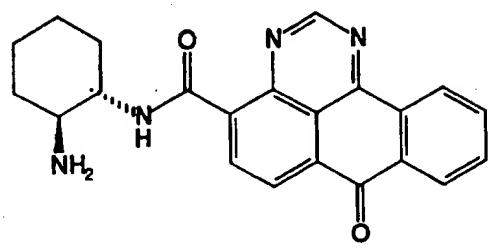


Preparation:

The product of example 6 (0.037, 0.1 mmol) was treated with 0.114 g (1 mmol) of (1R,2R)-(-)-1,2-diaminocyclohexane and the reaction mixture was

chromatographed (method A, step 2) to afford 0.043 g (90%) of the title compound: mp 208-212°C; APCIMS *m/z* 483 (M+1).

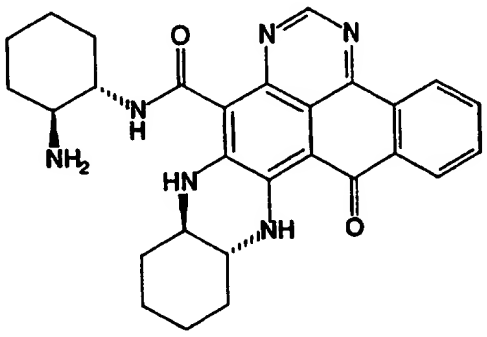
EXAMPLE 10



Preparation:

0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole and 0.114 g (1 mmol) of (1S,2S)-(+)-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 1) to afford 0.223 g (60%) of the title compound: ^{1}H NMR (300 MHz, DMSO-d_6) d 10.21 (d, J = 8.82, 1H), 9.66 (s, 1H), 8.88 (d, J = 7.72, 1H), 8.82 (d, J = 7.65, 1H), 8.65 (d, J = 7.64, 1H), 8.36 (d, J = 7.64, 1H), 7.99-8.07 (m, 2H), 7.90-7.97 (m, 1H), 4.03-4.17 (m, 1H), 3.10-3.25 (m, 1H), 1.95-2.10 (m, 2H), 1.70-1.85 (m, 2H), 1.40-1.70 (m, 2H), 1.30-1.40 (m, 2H); APCIMS *m/z* 373 (M+1).

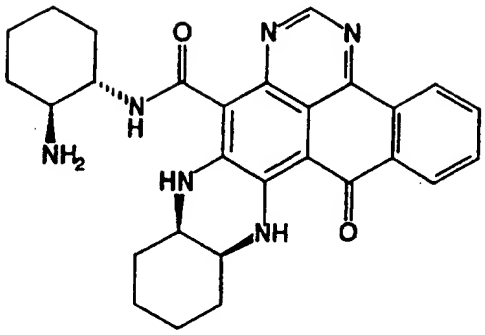
EXAMPLE 11



Preparation:

The product of example 10 (0.037, 0.1 mmol) was treated with 0.114 g (1 mmol) of (1R,2R)-(-)-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 2) to afford 0.038 g (78%) of the title compound: mp 190-193°C; ^{1}H NMR (300 MHz, DMSO-d_6) d 12.05 (s, 1H),11.66 (d, J = 8.37, 1H) 10.88 (s, 1H) 9.12 (s, 1H), 8.89 (d, J = 7.76, 1H), 8.40 (d, J = 7.75, 1H), 7.98 (brs, 2H), 7.90-7.95, (m, 1H), 7.82-7.87 (m, 1H), 4.00-4.09 (m, 1H), 3.34-3.42 (m, 1H), 3.10-3.26 (m, 2H), 2.18-2.27 (m, 1H), 1.98-2.10 (m, 3H), 1.73-1.90 (m, 4H), 1.30-1.70 (m, 8H); APCIMS *m/z* 483 (M+1).

EXAMPLE 12

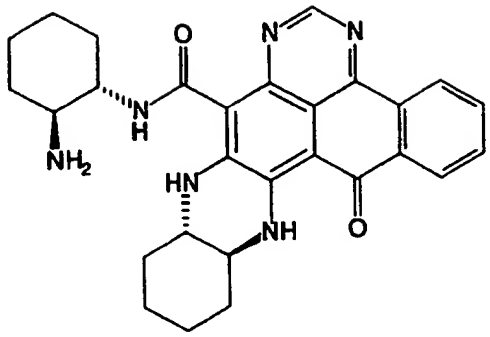


^C

Preparation:

The product of example 10 (0.037, 0.1 mmol) was treated with 0.114 g (1 mmol) of cis-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 2) to afford 0.035 g (73%) of the title compound: mp 185-187°C; ^{1}H NMR (300 MHz, DMSO-d_6) d 12.29 (s, 1H), 11.72 (s, 1H), 11.01 (s, 1H), 9.13 (s, 1H), 8.93 (d, J = 7.49, 1H), 8.45 (d, J = 7.61, 1H), 8.00 (brs, 3H), 7.82-7.97 (m, 2H), 3.80-4.40 (m, 3H), 3.10-3.40 (m, 1H), 1.20-2.20 (m, 16 H); APCIMS *m/z* 483 (M+1).

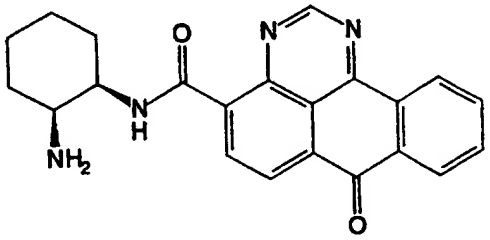
EXAMPLE 13



Preparation:

The product of example 10 (0.037, 0.1 mmol) was treated with 0.114 g (1 mmol) of (1S,2S)-(+)-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 2) to afford 0.037 g (77%) of the title compound: mp >250°C; APCIMS *m/z* 483 (M+1).

EXAMPLE 14

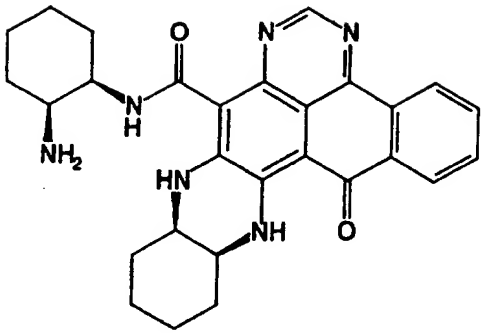


Preparation:

0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole and 0.114 g (1 mmol) of cis-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 1) to afford 0.300 g (80.6%) of the title compound: mp 172-175 °C; ^{1}H NMR (300 MHz, DMSO-d_6) d 10.89 (d, J = 8.82, 1H), 9.71 (s, 1H), 8.90 (d, J = 7.71, 1H), 8.85 (d, J = 7.30, 1H), 8.63 (d, J = 7.67, 1H), 8.33 (d, J = 7.28, 1H), 7.88-8.05 (m, 5H), 4.62-4.67 (m, 1H), 3.35-3.53 (m,1H), 1.75-1.95 (m, 4H), 1.55-1.75 (m, 3H), 1.35-1.55 (m, 1H); APCIMS *m/z* 373 (M+1).

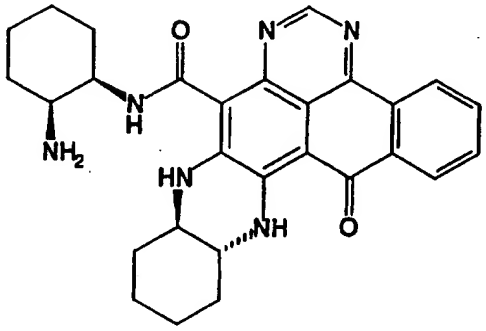
EXAMPLE 15

Preparation:



The product of example 14 (0.037g, 0.1 mmol) was treated with 0.114 g (1 mmol) of cis-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 2) to afford 0.024 g (50%) of the title compound: mp 146-150 °C; APCIMS *m/z* 483 (M+1).

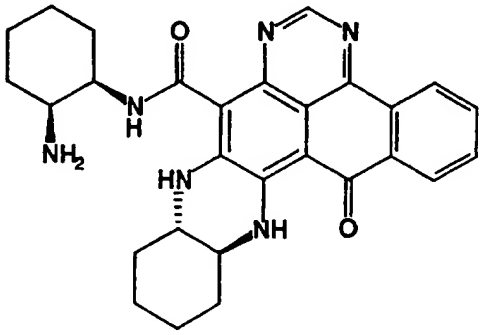
EXAMPLE 16



Preparation:

The product of example 14 (0.037g, 0.1 mmol) was treated with 0.114 g (1 mmol) of (1R,2R)-(-)-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 2) to afford 0.034 g (66%) of the title compound: mp 238-242°C; APCIMS *m/z* 483 (M+1).

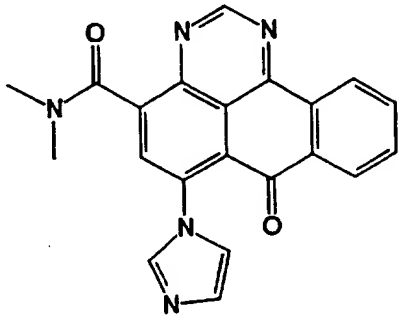
EXAMPLE 17



Preparation:

The product of example 14 (0.037g, 0.1 mmol) was treated with 0.114 g (1 mmol) of (1S,2S)-(+)-1,2-diaminocyclohexane and the reaction mixture was chromatographed (method A, step 2) to afford 0.036 g (75%) of the title compound: mp 180-183°C; APCIMS *m/z* 483 (M+1).

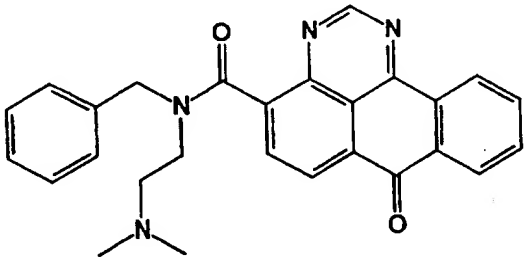
EXAMPLE 18



Preparation:

0.276 (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole in DMF and the reaction mixture was chromatographed (method B) to afford 0.012 g (3.2%) of the title compound: mp 173-175°C; APCIMS *m/z* 370 (M+1).

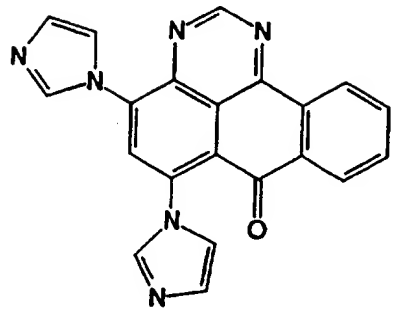
EXAMPLE 19



Preparation:

0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole and 0.178 g of N'-benzyl-N,N-dimethylethylenediamine, and the reaction mixture was chromatographed (method A, step 1) to afford 0.283 g (65%) of the title compound: mp 74-76°C; APCIMS *m/z* 437 (M+1).

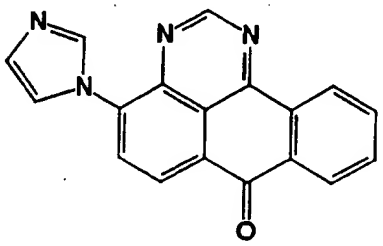
EXAMPLE 20



Preparation:

0.276 (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole and the reaction mixture was chromatographed (method B) to afford 0.020 g (5.5%) of the title compound: mp 128-132°C; APCIMS *m/z* 365 (M+1).

EXAMPLE 21

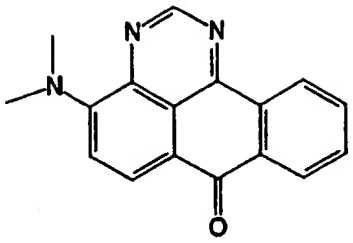


Preparation:

0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole and the reaction mixture was chromatographed (method B) to afford 0.050 g (16.8%) of the title compound: mp 142-144°C; ^{1}H NMR (300 MHz, DMSO-d_6) d 9.62 (d, J = 1.98, 1H), 9.16 (s, 1H), 8.85 (d, J = 7.76, 1H), 8.61-8.67 (m, 1H), 8.46-8.51 (m, 1H), 8.34 (d, J = 7.68, 1H), 7.96-8.04 (m, 1H), 7.88-7.95 (m, 1H), 7.63 (s, 1H); APCIMS *m/z* 299 (M+1).

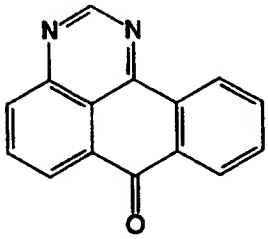
EXAMPLE 22

Preparation:



0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole in DMF and the reaction mixture was chromatographed (method B) to afford 0.008 g (2.6%) of the title compound:mp >250°C; ^{1}H NMR (300 MHz, DMSO-d_6) d 9.58 (s, 1H), 8.65 (dd, J = 7.76, 1.06, 1H), 8.55 (d, J = 7.44, 1H), 8.34 (dd, J = 7.68, 1.10, 1H), 8.15 (d, J = 7.43, 1H), 7.96-8.03 (m, 1H), 7.86-7.95 (m, 1H), 3.15 (s, 3H), 2.76 (s, 3H); APCIMS *m/z* 304 (M+1).

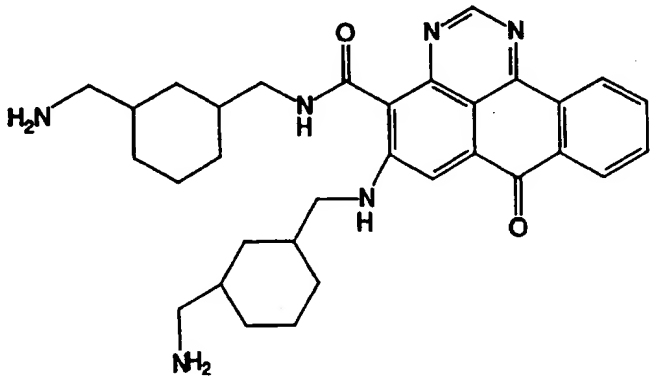
EXAMPLE 23



Preparation:

0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole and the reaction mixture was chromatographed (method B) to afford 0.080 g (34%) of the title compound: mp >250°C; APCIMS *m/z* 233 (M+1).

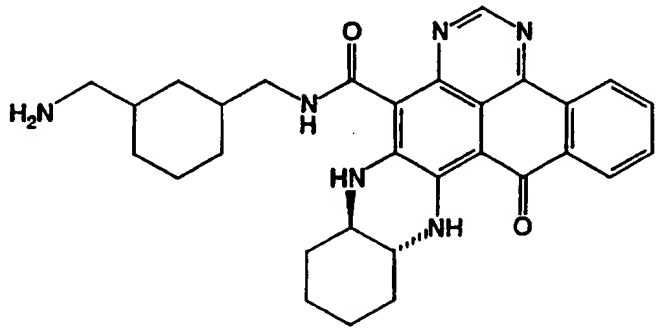
EXAMPLE 24



Preparation:

0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole and 0.142 g (1 mmol) of 1,3-cyclohexanebis (methylamine), and the reaction mixture was chromatographed (method C) to afford 0.062 g (11%) of the title compound: mp 192-194°C; APCIMS *m/z* 541 (M+1).

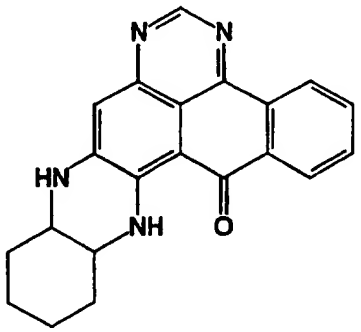
EXAMPLE 25



Preparation:

0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole, 0.142 g (1 mmol) of 1,3-cyclohexanebis (methylamine), and 0.114 g (1 mmol) of (1S,2S)-(+)-1,2-diaminocyclohexane, and the reaction mixture was chromatographed (method C) to afford 0.015 g (2.9%) of the title compound: mp 193-195°C; APCIMS *m/z* 511 (M+1).

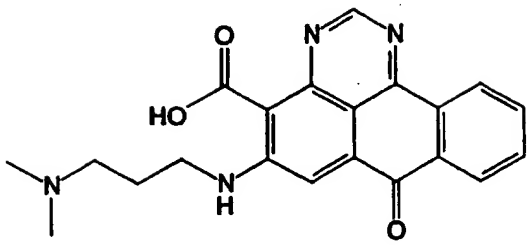
EXAMPLE 26



Preparation:

0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.114 g (1 mmol) of trans-1,2-diaminocyclohexane, and the reaction mixture was chromatographed (method D) to afford 0.020 g (5.8%) of the title compound: mp 198-200°C; APCIMS *m/z* 343 (M+1).

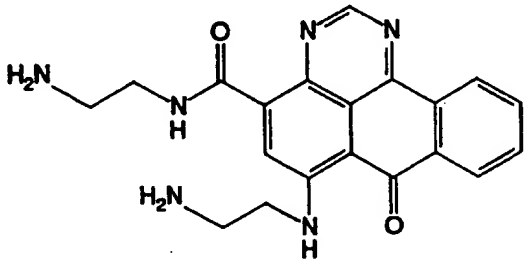
EXAMPLE 27



Preparation:

0.276 g (1 mmol) of 7-oxo-7H-benzo[e]perimidine-4-carboxylic acid was treated with 0.162 g (1 mmol) of 1,1'-carbonyldiimidazole, and 0.187 g (1 mmol) of 3,3'-iminobis(N,N-dimethylpropylamine), and the reaction mixture was chromatographed (method C) to afford 0.015 g (4%) of the title compound: mp 188-190°C; APCIMS *m/z* 376 (M+1).

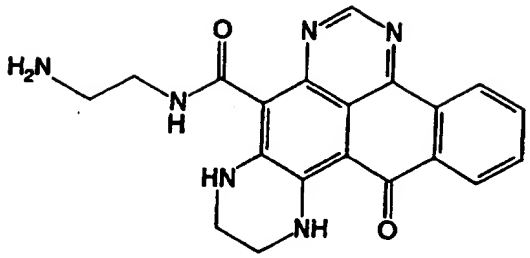
EXAMPLE 28



Preparation:

The product of Example 1 (0.031, 0.1 mmol) was treated with 0.62 g (1 mmol) of ethylene diamine and the reaction was chromatographed (method A, step 2) to afford 0.11 g (29%) of the title compound: mp >250°C; NMR (300 MHz, MeOH-d_4) d 9.00 - 9.15 (s, 1H), 8.55 - 8.90 (d, 1H), 8.10 - 8.40 (m, 2H), 7.55 - 7.90 (m, 2H), 3.55 - 4.00 (m, 4H), 3.25 - 3.45 (m, 4H); APCIMS *m/z* 378 (M+1).

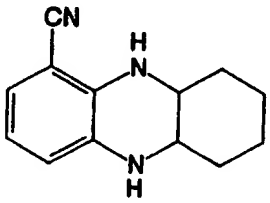
EXAMPLE 29



Preparation:

The product of Example 1 (0.031, 0.1 mmol) was treated with 0.60 g (1 mmol) of ethylene diamine and the reaction was chromatographed (method A, step 2) to afford 0.10 g (26%) of the title compound; mp 155-157°C; ^{1}H NMR (300 MHz, MeOH-d_4) d 9.00 - 9.15 (s, 1H), 8.35 - 8.45 (d, 1H), 8.20 - 8.35 (d, 1H), 7.75 - 7.90 (m, 2H), 3.95 - 4.05 (m, 2H), 3.25 - 3.65 (m, 6H); APCIMS *m/z* 374 (M+1).

EXAMPLE 30

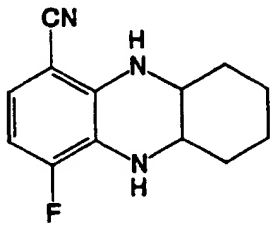


Preparation:

To a mixture of 1mL each of 0.1 M solution of 2, 3-difluorobenzonitrile and 1,2 diaminocyclohexane in DMSO was added 100 mg of KF/alumina and 10 mg of 18-crown-6, and the reaction mixture stirred at 120°C for 14 h. After workup the crude was loaded on a silica gel column and the products isolated by flash

chromatography: mp 120-122 °C; ^{1}H NMR(300 MHz, $CDCl_3$): 6.70 - 6.75(dd, 1H, Ar), 6.45 - 6.55(dd, 1H, Ar), 6.40 - 6.45(t, 1H, Ar), 4.15 - 4.35(s-br, 1H, NH), 3.50 -3.95(s-br, 1H, NH), 2.95 - 3.05(m, 1H), 2.60 - 2.95(m, 1H), 1.55 - 2.00(m, 4H), 1.05 -1.45(m, 4H); APCIMS *m/z* 214 (M+1).

EXAMPLE 31



Preparation:

To a mixture of 1mL each of 0.1 M solution of 2, 3, 4-trifluorobenzonitrile and 1,2 diaminocyclohexane in DMSO was added 100 mg of KF/alumina and 10 mg of 18-crown-6, and the reaction mixture stirred at 120°C for 14 h. After workup the crude was loaded on a silica gel column and the products isolated by flash chromatography: mp 163-167°C; ^{1}H NMR(300 MHz, $CDCl_3$): 6.50 - 6.65(d, 1H, Ar), 5.90 - 6.10(d, 1H, Ar), 3.85 - 4.05(s-br, 1H, NH), 3.55 - 3.65(s-br, 1H, NH), 2.70 - 2.85(m, 1H), 2.55 - 2.65(m, 1H), 1.40 - 1.85(m, 4H), 0.95 - 1.25(m, 4H); APCIMS *m/z* 232 (M+1).

BIOLOGICAL EVALUATION

EXAMPLE 32

Isolation of CRF membrane receptors

Cell Culture

Human embryonic kidney 293-EBNA cells stably transfected with cDNA for rat CRF_1 (Chang et al., 1993) or mouse CRF_{2b} (Kishimoto et al., 1995) receptors were generously provided by Dr. M.G. Rosenfeld, Howard Hughes Medical Institute, University of California at San Diego. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum, 5% neonatal calf serum, 100U/ml Penicillin-G/Streptomycin, 2 μg/ml Fungizone™ and 10U/ml Hygromycin-B.

Membrane Preparation

Fresh or frozen 293-EBNA cells were homogenized in approximately 50 ml of homogenization buffer (buffer A) containing 50 mM Tris, 2 mM EGTA and 0.32M Sucrose (pH 7.4) using an Ultra-Turax homogenizer (Tekmar Company, Cincinnati, OH) at 80% maximal setting three times for 10 sec. Cell pellets were centrifuged at 4°C at 1000x*g* for 10 min in a Beckman GS-6R centrifuge. Pellets were resuspended in buffer A, homogenized and centrifuged as described above. Pooled supernatants were transferred to centrifuge bottles (Beckman) and centrifuged at 4 °C at 20,000 x*g* for 30 min in a Beckman J2-HS centrifuge. Cell pellets were resuspended in

buffer A and again were centrifuged at 4°C at 20,000 x*g* for 30 min. Cell pellets were resuspended in buffer A and stored at -70°C in aliquots of 2.5-5 mg/ml total membrane protein. Total membrane protein was determined by a BCA kit (Pierce, Rockford, IL).

Radioligand Binding Assays

Membranes were thawed and resuspended in binding assay buffer containing: 50 mM HEPES (pH 7.4), 2 mM EGTA, 0.1% BSA, 5 mM $MgCl_2$ and 0.01% bacitracin. Membranes (15-25 μg protein/tube) were incubated in duplicate with ^{125}I-Tyr^0-oCRF (25,000 CPM/tube; 2200 Ci/mmol) and various compounds for 1 hr at room temp. Compounds were dissolved in 100% DMSO and were tested in binding assay buffer containing final concentrations of 1 pM-100 μM in 10% DMSO final. Nonspecific binding was determined in the presence of either 1 μM oCRF or 100 nM sauvagine. Reactions were terminated by rapid filtration onto Whatman GF/C filters (Brandel) soaked with 0.1% polyethylenimine by use of a 48-well cell harvester (Brandel). Filters were washed three times with ice-cold wash buffer containing: 50 mM $NaPO_4$ (pH 7.4), 0.9% NaCl, 2 mM $MgCl_2$, 0.02% NaN_3 and 0.01% Triton X-100. Filters were counted on a Packard Cobra gamma counter.

EXAMPLE 33

Cyclic AMP Determination

Approximately two million 293-EBNA Cells/tube expressing either CRF_1 or CRF_{2b} receptors were incubated in triplicate at 37 °C in a shaking water bath for ten min in cAMP generation buffer containing: 10 mM HEPES (pH 7.4), 30 mM NaCl, 4.7 mM KCl, 2.5 mM NaH_2PO_4, 1. 4 mM $MgCl_2$, 1 mM EGTA, 3 mM Glucose, 0.2% BSA and 50 μM 1-methyl-3-isobutylxanthine (IBMX). Cells then were incubated in suspension at 37 °C in siliconized glass 12 x 75 mm tubes containing various concentrations of antagonists for 25 min. Compounds were first dissolved in 100% DMSO and were further diluted in cAMP generation buffer to yield final concentrations between 1 pM and 100 μM in 1% DMSO. Cells were then stimulated for five min with either 1 nM or 3 nM oCRF (found to be half-maximal for CRF_1 or CRF_{2b} receptors, respectively) for cells containing CRF_1 or CRF_{2b} receptors, respectively. In order to test for agonist activity, compounds were tested alone or in the presence of forskolin (1-10 μM) for their ability to stimulate cAMP formation. Reactions were stopped by immediately centrifuging the cells 3 min at 500 x *g*. Cell pellets were lysed with 0.5 ml of 0.1N HCl, bath sonicated and centrifuged at 2000 x*g*. Supernatants were transferred to clean glass 12 x 75 mm test tubes and centrifuged in a Speed-Vac under high heat for 2 h. Dried cell extracts were reconstituted with sodium acetate buffer (pH 6.2, supplied with kit) and analyzed for cAMP by use of a RIA kit (DuPont-New England Nuclear).

EXAMPLE 34

Assessment of *in vivo* biological activity

A variety of *in vivo* techniques can be utilized for assessment of biological activity. These include and are not limited by: the Acoustic Startle Assay, Cold Swim, physical restraint, ether inhalation, Elevated Plus-maze Test,

Stair climbing test, stress- and drug-induced anorexia or Chronic Administration Test as outlined (Heinrichs et al., Ann NY Acad Sci 771:92-104, 1995; Berridge and Dunn, Brain Res Rev 15:71, 1990.). These tests can be performed on rodents and small animals.

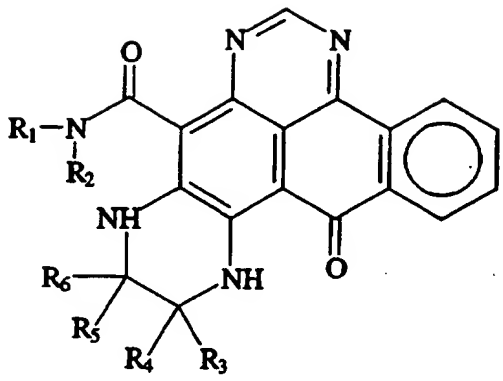
EXAMPLE 35

Data Analysis

Binding and functional cAMP data were analyzed with Prism™ (GraphPad, San Diego, CA), a computer graphics and statistics program. IC_50 values and Hill slopes for radioligand binding experiments were generated by nonlinear regression using Prism™.

WHAT IS CLAIMED IS:

1. A compound having the structure



wherein

R1 is

(a) H;

(b) (C_1-C_8) alkyl;

(c) (C_1-C_8)-NH-(C_1-C_4)alkyl;

(d) (C_1-C_8)-NH-((C_1-C_4)alkyl)$_2$;

(e) (C_1-C_8)cycloalkyl;

(f) (C_1-C_8)cycloalkyl-NH_2;

(g) (C_1-C_8)cycloalkyl-NH-(C_1-C_4)alkyl;

(h) (C_1-C_8)cycloalkyl-NH-((C_1-C_4)alkyl)$_2$;

(i) (C_1-C_3)alkyl-(C_3-C_8)cycloalkyl-(C_1-C_3)alkyl-NH_2;

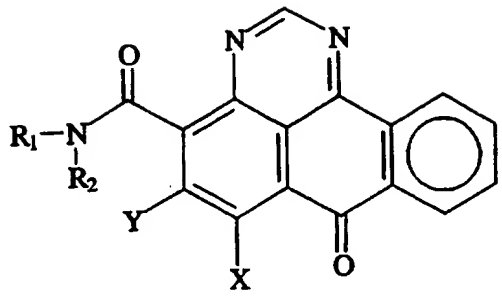
(j) (C_1-C_3)alkyl-(C_3-C_8)cycloalkyl-(C_1-C_3)alkyl-NH-(C_1-C_3)alkyl;

(k) NH-(C_1-C_8)cycloalkyl-$NHNH_2$;

R_2 is H or (C_1-C_4)alkyl; and

R_3, R_4, R_5, and R_6 are independently H; COOH; (C_1-C_8)alkyl halide; or (C_1-C_8)alkyl ester, including enantiomers and stereoisomers of these compounds, and pharmaceutically acceptable salts thereof.

2. A compound having the structure



wherein R_1 and R_2 are as defined in Claim 1; and

X and Y are independently

(a) H;

(b) NH-(C_1-C_6) alkyl, straight or branched C-C chain;

(c) N(C_1-C_6)$_2$ alkyl, straight or branched C-C chain;

(d) NH(C_1-C_6) alkyl-NH_2, straight or branched C-C chain;

(e) N(C_1-C_6) alkyl-NH_2)$_2$, straight or branched C-C chain;

(f) amidine;

(g) mono- and di-substituted amidine;

(h) guanidine;

(i) mono- or di-substituted guanidine;

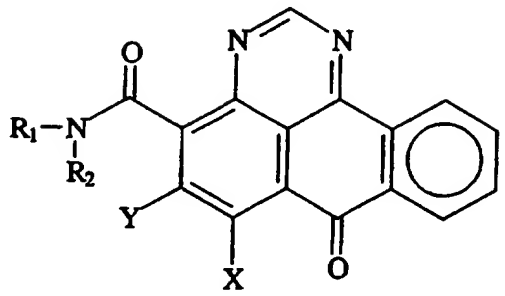
(j) NHCO-(C_1-C_6)alkyl, NHCO-aryl, NHCO-heteroaryl;

(k) NHCO-O-(C_1-C_6)alkyl, NHCO-O-aryl, NHCO-O-heteroaryl; or

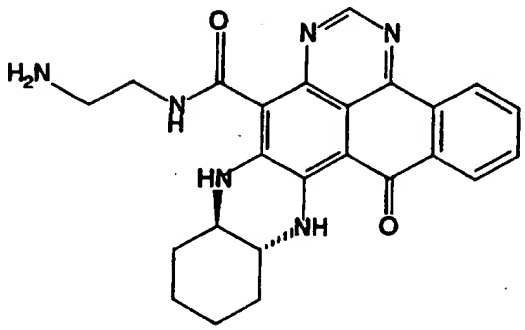
(l) NHCONH(C_1-C_6)alkyl, NHCONH-aryl, or NHCONH-heteroaryl; or X and Y taken together are NH-$(CH_2)_2$-NH_2 and X and Y are joined to the benzoperimidine group to form a 5,6-fused piperazine ring,

including enantiomers and stereoisomers of these compounds, and pharmaceutically acceptable salts thereof.

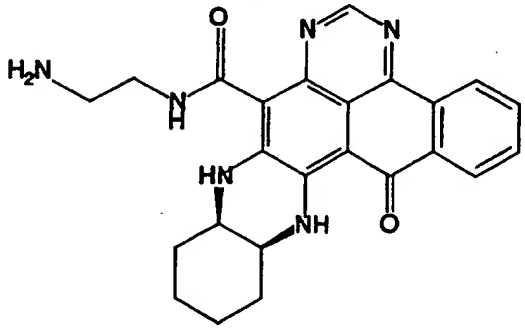
3. A compound according to Claim 1 or 2 of the formula



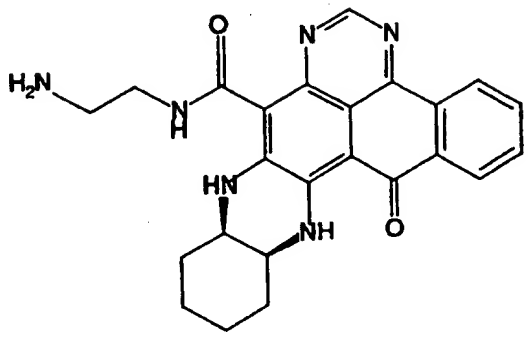
4. A compound according to Claim 1 or Claim 2 of the formula:



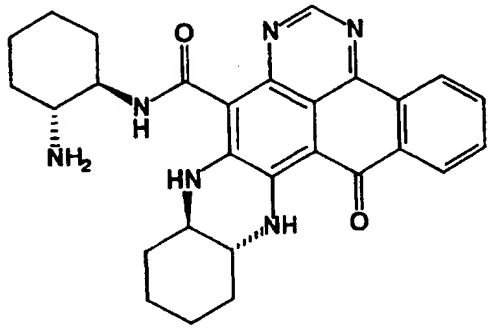
5. A compound according to Claim 1 or Claim 2 of the formula:



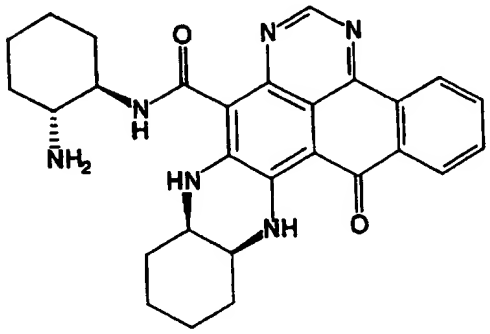
6. A compound according to Claim 1 or Claim 2 of the formula:



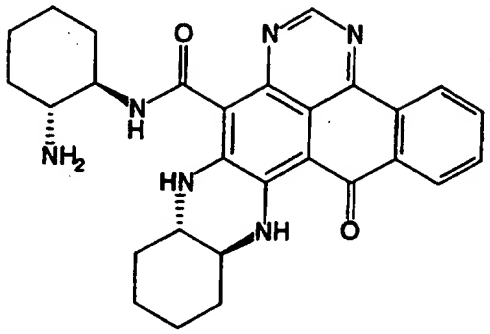
7. A compound according to Claim 1 or Claim 2 of the formula:



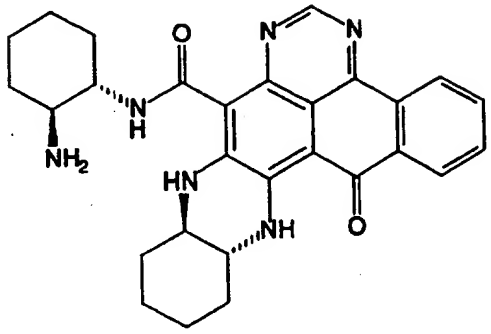
8. A compound according to Claim 1 or Claim 2 of the formula:



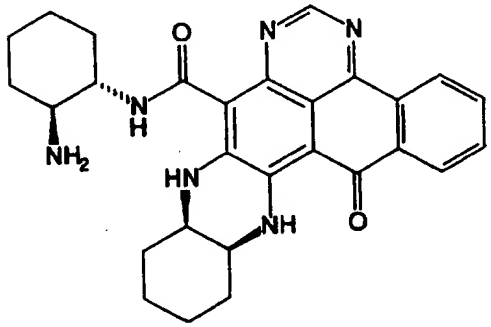
9. A compound according to Claim 1 or Claim 2 of the formula:



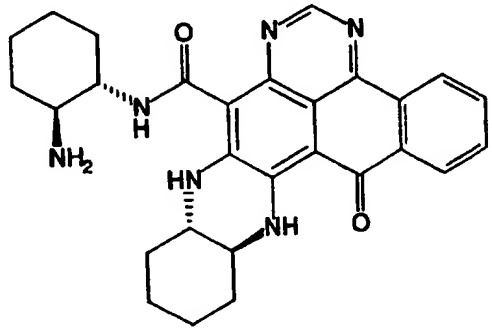
10. A compound according to Claim 1 or Claim 2 of the formula:



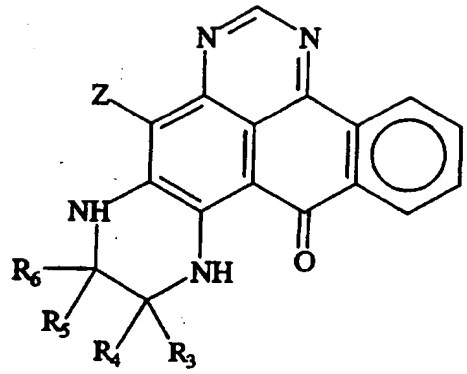
11. A compound according to Claim 1 or Claim 2 of the formula:



12. A compound according to Claim 1 or Claim 2 of the formula:



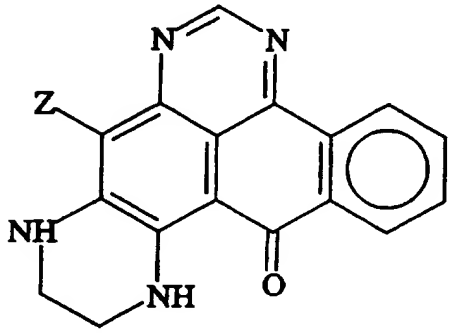
13. A compound of the formula



wherein $R_{3-R}6$ are as defined in Claim 1; and

Z is a carboxyl amide.

14. A compound of the formula



wherein Z is a carboxyl amide.

15. A compound according to Claim 14 wherein Z is a carboxyl amide having the formula $C(O)NH\text{-}R_2$, wherein R_2 is as defined in Claim 1.

16. A pharmaceutical formulation comprising, as an active component, a compound according to any of Claims 1-15, in a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14955

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07D 239/00, 239/70; A61K 31/495, 31/50, 31/505
US CL :544/245, 248; 514/250, 257

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 544/245, 248; 514/250, 257

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chem. abstr., Vol. 118, No. 19, 10 May 1993 (Columbus, OH, USA), page 22, column 1, the abstract No. 118:182772p, ANTONINI, I. et al. 'Synthesis of 7-oxo-7H-benzo[e]perimidine-4-carboxamides as potential antitumor drugs.' Farmaco 1992, 47(11), 1385-93 (Eng).	2, 3
A	Chem. abstr., Vol. 123, No. 10, 04 September 1995 (Columbus, OH, USA), page 1135, column 1, the abstract No. 123:127575e, HIROSE, H. 'Electrophotographic photoreceptor with superior electrophotographic characteristics.' JP 07,160,021 [95,160,021], 23 June 1995.	2, 3

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 05 OCTOBER 1997	Date of mailing of the international search report 02 DEC 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DEEPAK RAO Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14955

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Chem. abstr., Vol. 118, No. 5, 01 February 1993 (Columbus, OH, USA), page 682, column 1, the abstract No. 118:38872d, STEFANSKA, B. et al. '6-[(Aminoalkyl)amino]-substituted 7H-benzo[e]perimidin-7-ones as novel antineoplastic agents. Synthesis and biological evaluation.' J. Med. Chem. 1993, 36(1), 38-41 (Eng).	2, 3
A	Chem. abstr., Vol. 86, No. 15, 11 April 1977 (Columbus, OH, USA), page 552, column 1, the abstract No. 86:114448h, MORIGA, H. et al. 'Plastic thermistor compositions.' JP 76 45,616, 04 December 1976.	2, 3
A	Chem. abstr., Vol. 83, No. 1, 07 July 1975 (Columbus, OH, USA), pages 854-855, column 2 and top of column 3, the abstract No. 10137c, POPOV, S. I. et al. 'Substituted phenanthrapyrimidines.' U.S.S.R. 457,702, 25 January 1973.	2, 3
A	Chem. abstr., Vol. 80, No. 4, 28 January 1974 (Columbus, OH, USA), page 42, column 1, the abstract No. 15740s, SAENGER, D. et al. 'Light-degradable thermoplastics.' Ger. Offen. 2,209,139, 30 August 1973.	2, 3
A	US 4,927,820 A (SHUTSKE et al.) 22 May 1990, column 1, formula (I), lines 7-14.	2, 3
A	US 4,001,170 A (WICK) 04 January 1977, column 1, formula (I), lines 10-25.	2, 3
A	US 3,939,162 A (ELSER et al.) 17 February 1976, column 1, formula (I), lines 6-13.	2, 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14955

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 16
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.